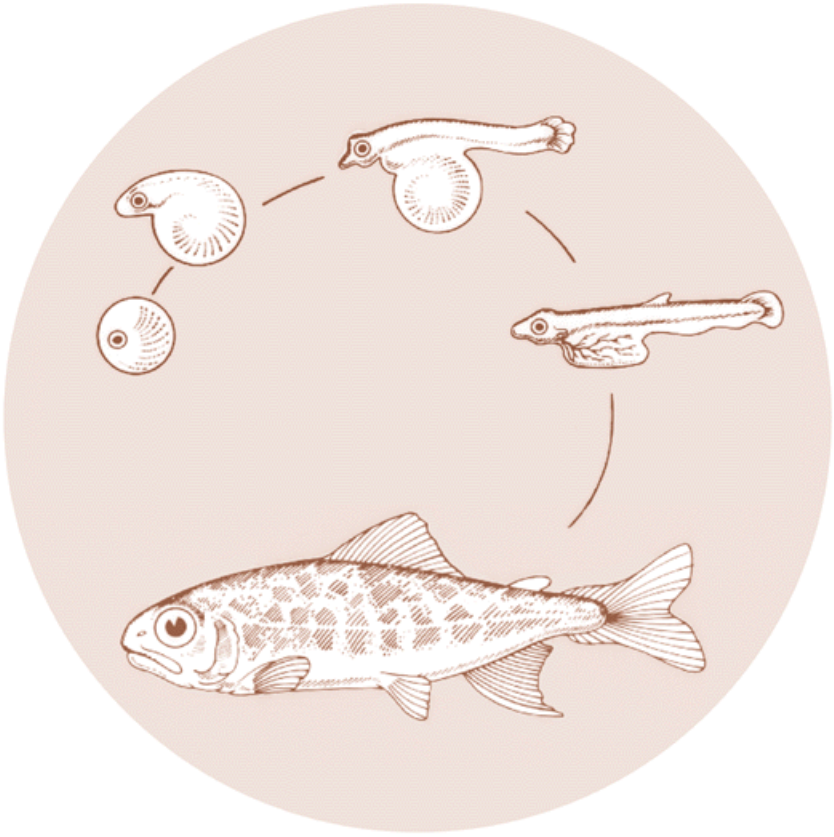
October 1996

PHYSIOLOGICAL ASSESSMENT AND BEHAVIORAL INTERACTION OF WILD AND HATCHERY JUVENILE SALMONIDS:

The Relationship Of Fish Size And Growth To Smoltification In Spring Chinook Salmon



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PHYSIOLOGICAL ASSESSMENT AND BEHAVIORAL INTERACTION OF WILD AND HATCHERY JUVENILE SALMONIDS: THE RELATIONSHIP OF FISH SIZE AND GROWTH TO SMOLTIFICATION IN SPRING CHINOOK SALMON

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EXECUTIVE SUMMARY

Experiments were performed to determine the relative influence of size and growth rate on downstream migratory disposition and physiology in yearling spring chinook salmon (*Oncorhynchus tshawytscha*) smolts. A group of juvenile chinook salmon was size graded into small and large categories with half the fish in each group reared at an elevated temperature, resulting in four distinct treatment groups: Large Warm (LW), Large Cool (LC), Small Warm (SW), and Small Cool (SC). Fish from warm-water treatment groups displayed significantly higher growth rates than cool-water groups. Fish were tagged and released into a natural creek where downstream movement was monitored. For each of the two releases, fish that migrated past a weir within the first 5 days postrelease had significantly higher spring growth rates than fish that did not migrate within that period. Significant differences in length for the same fish were only found in the second release. Also for the second release, fish from the warm water treatment groups were recovered in higher proportions than fish from cool water groups. The results indicate that increased growth rate in the spring has a positive relation to downstream migratory disposition. Furthermore, there is a relation between smolt size and migration; however, this relation is weaker than that found between growth rate and migration.

Separate groups of juvenile chinook salmon in the four groups (LW, LC, SW, and SC) were sampled for analysis of physiological changes during smoltification. Temporal increases in insulin-like growth factor-I (IGF-I) were found in all groups through the spring. Plasma IGF-I levels were significantly higher in warm-water groups than cool-water groups from late March through May. Size itself appeared to have little relation to plasma IGF-I levels. Simple regression showed a significant relation between plasma IGF-I and growth ($P < .001$, $R^2 = .69$). No differences were found between treatment groups in other physiological parameters assessed (plasma thyroxine, gill Na^+-K^+

ATPase, liver glycogen. body lipid). Results suggest that growth rate rather than fish size was a more physiologically relevant aspect of smoltification and subsequent smolt-to-adult survival.

We observed a relatively strong effect of growth rate on downstream migratory tendency, in contrast to its relatively modest effect on physiology. This finding is significant for both the biology of smolt transformation/development and hatchery management Rapid and directed downstream migration is a most essential element of smoltification. Stimulation of growth of hatchery-reared salmonids during the Parr-smolt transformation may improve smolt quality by 1) improving downstream migration and 2) improving seawater tolerance through stimulation of the GH-IGF-I axis. We suggest that hatcheries do not focus on absolute size as a criterion for fish production, but instead develop production schemes which emphasize achieving high rates of fish growth prior to release. However, it is unclear whether simple increases in ration at a constant temperature would also have a stimulatory effect, especially as many hatcheries already feed rations designed to produce optimal growth. A larger-scale test of the relative effects of water temperature and feeding rate and their subsequent effects on growth and smolt performance is warranted.

INTRODUCTION

The pat-r-to-smolt transformation (smoltification) of juvenile salmonids involves a suite of morphological, behavioral and physiological changes that both allow and stimulate the transition from fresh water to seawater (Hoar 1988). Seasonally changing photoperiod has been clearly shown to entrain smolt development (Zaugg and Wagner 1973, Wagner 1974). Furthermore, there is a seasonal window for smolt release from hatcheries that results in the highest adult return (Bilton et al. 1982). For the last several decades research in many laboratories has focused on understanding the factors responsible for controlling smoltification and improving the quality of smolts released by public hatcheries.

High-quality smolts can be defined operationally as juvenile salmonids that show rapid downstream migration, long-term growth, and survival to adulthood. High-quality smolts with higher survival rates should improve efficiency in public hatcheries by reducing the number of smolts that must be released in order to maintain adult production levels. Reducing the number of smolts released by hatcheries should reduce hatchery-rearing costs and lessen any negative impacts of hatchery fish on naturally tearing fish. Producing high-quality smolts that migrate downstream rapidly should also reduce these impacts because rapidly migrating fish would interact less with wild salmonids. Furthermore, rapidly migrating fish would be less likely to stray and less likely to imprint on sites outside the hatchery. This would reduce introgression by minimizing the occurance of hatchery fish spawning with wild fish. Several hundred million smolts are released each year from hatcheries in the Pacific Northwest; improving smolt quality and migration is currently a major management goal (Schmitten et al. 1995).

Smolt size has been suggested as a factor which can influence migratory behavior and subsequent survival to adulthood. Large smelts have been found to migrate sooner than smaller fish in natural populations (Irvine and Ward 1989, Bohlin et al. 1993).

Several studies have shown that larger smolts within a year class tend to survive to adult at a higher rate than smaller fish (Ward and Slaney 1988, Ward et al. 1989, Henderson and Cass 1991). Similarly, large hatchery smolts have been shown to migrate sooner than their small cohorts (Ewing et al. 1984, Hansen and Jonsson 1985) and to have relatively greater survival to adulthood (Bilton et al. 1982, Martin and Wertheimer 1989). Several studies of Atlantic salmon (Salmo *salar)* have found a relation between size of smolts at release and adult returns (Virtanen et al. 1991, Farmer 1994).

These findings have resulted in management practices that promote the release of smolts at a relatively large size (Mahnken et al. 1982). However, larger smolt size at release does not always correlate with high adult survival. Zaugg (1989) demonstrated a relationship between smolt development, assessed by gill $Na^+ K^+$ ATPase activity, prior to release and adult return in subyearling chinook salmon. Yet, he did not find a relation between size at release and adult return. Virtanen et al. (1991) also found a relationship between physiological indicators of smoltification and adult return in Atlantic salmon.

In many of the studies examining smolt size at release and adult survival, the larger smolts were larger because of recent growth history. Thus, large smolts were also faster growing. A few studies have noted that growth rate may have a greater influence on smoltification than body size. Wagner et al. (1969) noted that fall chinook salmon *(Oncorhychus tshuwytschu)* exhibiting high growth rates showed better seawater tolerance than larger, slower-growing fish. Vamavskiy et al. (1992) measured RNA/DNA as an index of growth rate and found that faster-growing coho salmon smolts (0. *kisutch)* migrated through the estuary more quickly than slower-growing fish. Dickhoff et al. (1995) showed a relation between spring growth rate of hatchery spring chinook salmon smolts (0. *tshawytscha*) prior to release and subsequent adult return rates. Together these results led us to question whether past demonstrations of superior performance by large smolts were due to their physical size or to their recent growth

history, with fast growth leading to intensified physiological development and migratory performance.

These studies were undertaken to separately evaluate the influence of fish body size and growth rate on the Parr-smolt transformation of yearling chinook salmon. The progression of the parr-smolt transformation was measured by downstream migration and by changes in biochemical-endocrinological indices of smoltitication.

MATERIALS AND METHODS

Fish Rearing

Adult spring chinook salmon were captured prior to spawning in the Yakima River, a tributary of the Columbia River located in central Washington State. Gametes were stripped from adults captured near the spawning grounds and transported to a research hatchery in Seattle. Eggs and alevins were incubated in Heath trays.[1] Fry were transferred to 1.3-m circular tanks and reared according to standard hatchery techniques (Piper et al. 1982). In August 1993,20,000 subyearling fish were sorted into two size classes. Approximately 6,000 fish from small (mean length = 60 mm; se = 0.6) and large (mean length = 82.5 mm; se = 0.4) size categories were retained. Fish from each size category were distributed into twelve tanks at 500 fish/tank.

Fish were reared with seasonally changing water temperature under incandescent lights with a daily photoperiod that was adjusted weekly to simulate that of Seattle (latitude 48° N). Beginning in mid-February, water for four tanks from each size group was heated above ambient temperature (Fig. 1). This resulted in four experimental groups: SmallCool (SC), SmallWarm (SW), LargeCool (LC), and LargeWarm (LW), each contained in replicate tanks. Fish were fed Biodiet (Bioproducts Inc. Warrenton OR.) at levels corresponding to feed manufacturer's recommendations (1.2-2.5s body weight/day) for each size and temperature.

Individuals selected for migratory behavior tests were weighed and measured l-3 days prior to testing. Instantaneous growth rates for each fish ((lnL2 - lnL1)*(T2-T1)$^{-1}$ *lOO) were calculated (where Ll = length at tagging, L2 = length at release, T2-Tl = days between release and tagging). Four of the twelve tanks of two size and temperature

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1 **Reference to tradename does not imply endorsement by National Marine Fishcries Service, NOAA.**

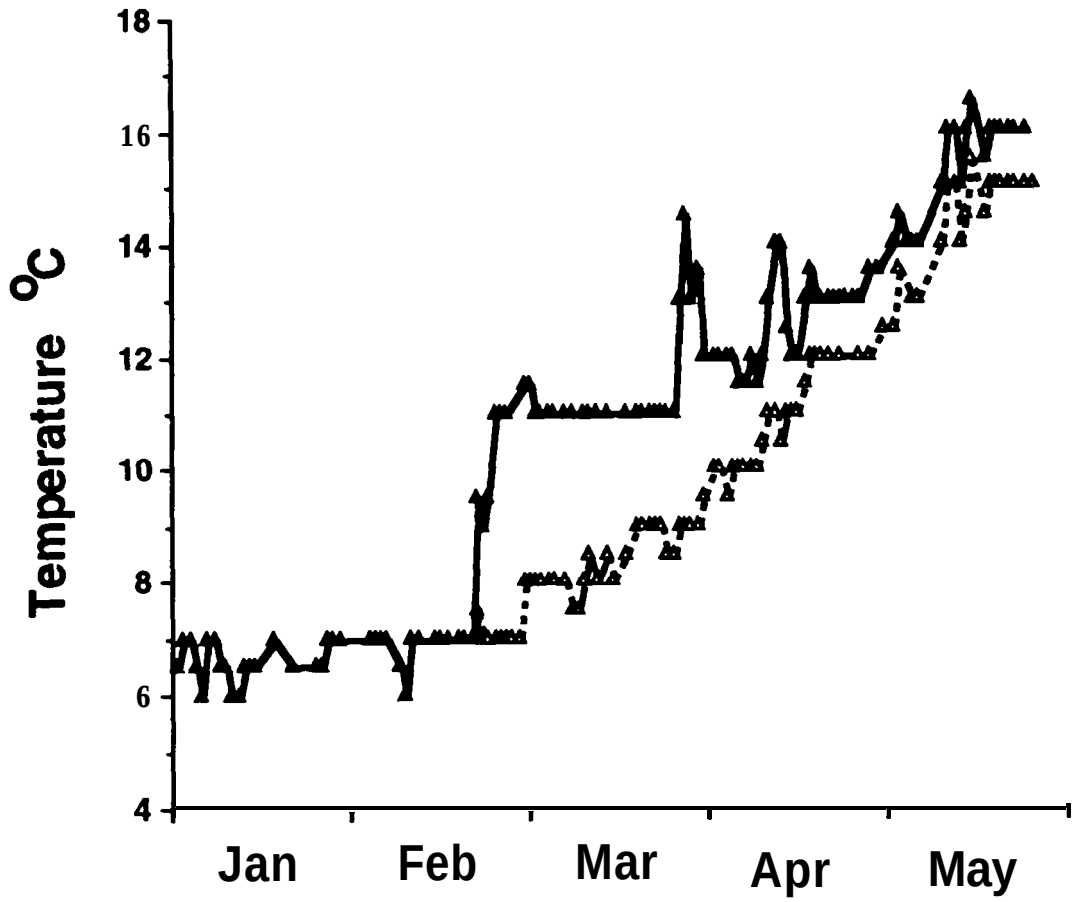

Figure 1. Water temperatures of experimental groups; ambient (open triangles - dashed line) and heated (filled triangles and solid line).

categories were used in the migration experiment, and the remaining eight were used in the physiology experiment.

Migration experiment

Passive integrated transponder tags (PIT tags, Destron) were implanted into 1,000 large and 1,000 small fish on 16 January 1994. Fish were maintained in 1.3-m-diameter circular tanks at 500 fish/tank. Water temperature was adjusted in one tank of large fish and one tank of small fish as described above.

Migrational behavior was tested on 25 March and 12 April 1994. On each date about 100 fish from each group were netted into a 1,000-L oxygenated transport tank for transfer to the test site by truck. Transfer from rearing site to release site took less than 3 hours. Fish were bucketed into the creek soon after arrival, and monitoring of movement began immediately.

Tests were conducted at Cooke Creek, a fourth order tributary of the Yakima River, located in central Washington. One PIT-tag detector (Biomark Inc. Boise ID.) was placed at the apex of each of two separate, V-shaped weirs located 1.0 (Weir 1) and 1.3 km (Weir 2) downstream from the release site. Cooke Creek has a moderate gradient and there were several riffle-pool sequences between Weir 1 and Weir 2. There were many riffle-pool sequences between the release site and Weir 1, including several large pools behind debris jams. Each of these pools was capable of holding several hundred fish at densities lower than those found at the hatchery. Indigenous fish in Cooke Creek included small rainbow (0. *mykiss)* trout and sculpin (spp.). Irrigation diversions in the lower reaches of the creek prevented passage of anadromous fish into the creek. Observations by snorkel-equipped divers indicated relatively little behavioral interaction between released salmon smolts and indigenous fishes (Jeff McMichael, Washington Department of Fish and Wildlife, 801 S. Ruby St., Ellensburg, WA 98926, Pers.

commun., May 1994). Creek discharge was monitored by measuring creek height daily at 5:00 p.m. (generally peak discharge).

Weirs consisted of 6.4-mm mesh hardware cloth stapled over 2.5-m by 1.1-m wood frames. Several panels were butted together to form the weir. Fish were monitored as they passed through a PVC pipe (15.2-cm diameter), the only downstream path through the weir. The time and date of passage for each individual fish was logged by the PIT-tag detector as fish passed downstream. At the second weir fish were routed into a plywood holding box, a trapping method which ensured that all fish were logged with 100% efficiency. Batteries and floppy discs in each interrogator were exchanged daily.

The Cooke Creek drainage lies at the base of the Wenatchee Mountains. Extensive snow fields on the flanks of this range are subject to rapid melting on warm spring days, and such conditions occurred approximately a week subsequent to each release. This resulted in flooding that precluded maintaining weir integrity. Flooding was a stochastic event that separated released fish into two groups: early migrating (EM), which passed weirs before flooding and were monitored, and non-detected (ND), which most likely passed the weir after flooding and were not monitored.

Differences in length and growth rate between groups prior to release were determined by one-way ANOVA, followed by Fisher's protected least significant difference test to determine differences between individual means. Differences in migratory tendency between groups (the number of fish from each group {LW, LC, SW, SC} which were monitored at the weir prior to flooding) were analyzed by chi-square. The relative growth rates prior to release and release length of EM and LM were similarly assessed with one-way ANOVA. Finally, relationships between release length or spring growth rate to migrational speed (hours from release to a weir) were examined by Spearman rank correlations. All analyses were conducted with Statview II statistical software (Brainpower Inc.. Cupertino CA.).

Physiology experiment

A monthly inventory of fish in all tanks was made beginning on 20 January 1994. Lengths and weights were measured from 60 fish per tank. Instantaneous growth rates (IG) for the period between inventories were calculated for each tank as described above. Outliers (2 sd > mean) were removed from length and weight data before analyses were made.

Beginning in mid-January and continuing on a biweekly schedule through May, six fish were killed from each tank (12 fish/treatment) to obtain physiological samples. Twelve fish at a time *were* netted from tanks and placed in 20-L buckets. Fish were placed one at a time into a lethal concentration (0.2 g/L) of tricaine methanesulfonate (MS-222). Fish were weighed and measured, the tail was cut, and blood was collected in heparinized glass tubes from the caudal peduncle. Blood was centrifuged for 3 minutes at 3000 x g, and plasma was removed, frozen, and stored at -800C. Gill tissue was removed from three arches and placed in a solution of sucrose, EDTA, and imidazole according to methods described by Zaugg (1982). and then frozen on dry ice and stored at -80°C. Livers were removed and immediately smashed and frozen between two pieces of dry ice. Liver chips were then placed in a well of a 24-well plate and stored at -800C. Fish carcasses were individually‘placed in plastic bags and stored frozen at -800C.

Fish in our experiment experienced mortality due to an epizootic of bacterial kidney disease (*Renibacterium salmoninarum*, BKD). Fish with obvious signs of BKD were not sampled. Furthermore, physiological data from fish that had low hematocrits (< 20%) were not included in any analysis.

Plasma IGF-I concentration was determined according to the methods described by Moriyama et al. (1994). Plasma T4 concentrations were determined according to the methods of Dickhoff et al. (1982). Gill ATPase activities were measured using the method of Schrock et al. (1994) and liver glycogen content was determined with the

method described by Plisetskaya et al. (1994). Whole body lipid was determined by the method of Soxhlet (AOAC, 1975) with lipid extracted with methylene chloride.

All data were first examined for differences between replicate tanks within treatments. Data for a treatment were pooled if no significant difference ($P > 0.05$) between replicates was detected in a two-way Analysis of Variance (ANOVA) examining date and replicate. Results were then examined using a three-way ANOVA with date, temperature, and size being the effects modeled. If significant effects were found, differences between individual means (either differences between treatments for a given date or differences between dates for a given treatment) were examined using one-way ANOVA followed by Fisher's protected least significant difference (PLSD). Results were considered significant at $P < 0.05$. Linear regression was used to examine the relationship between plasma hormone levels and instantaneous growth. Mean plasma hormone level found in fish from a single tank for one growth interval were regressed against instantaneous growth for that interval.

RESULTS

Migration Experiment

Average fish lengths at tagging and at release are shown in Figure 2. There were significant differences between groups for Release 1 ($F = 252.2$, $P = 0.0001$). LW fish were significantly larger than LC fish. which in turn were larger than both SW and SC fish. Groups of SC and SW were not significantly different from each other. Average lengths of each of the treatment groups for Release 2 were significantly different ($F = 87.7$. $P = 0.0001$).

Significant differences in growth rate were found among fish from the different treatments for Release 1 ($F = 25.4$, $P = 0.0001$; Fig. 3). The LW fish grew at significantly higher rates than the SW fish, which grew at a significantly higher rate than either cool water group (SC and LC). There were no significant differences between the SC and LC fish in growth rate. Both warm water groups (SW and LW) showed significantly higher growth rates than the cool water groups (SC and LC. $F = 18.3$, $P = 0.0001$) for Release 2. There was no significant difference in growth rate between groups reared at the same temperature.

The chronology of fish release and monitoring is shown in Figure 4. Of the 482 fish in Release 1, 150 were observed in the 6 days prior to the flooding that collapsed the weir. An additional 19 fish were monitored from Release 1 after the weir was installed the second time. These 19 fish were designated as Stanza B of Release 1 and are only considered in results when specifically mentioned. In the 5 days of monitoring conducted for Release 2. 170 of 324 fish released were observed.

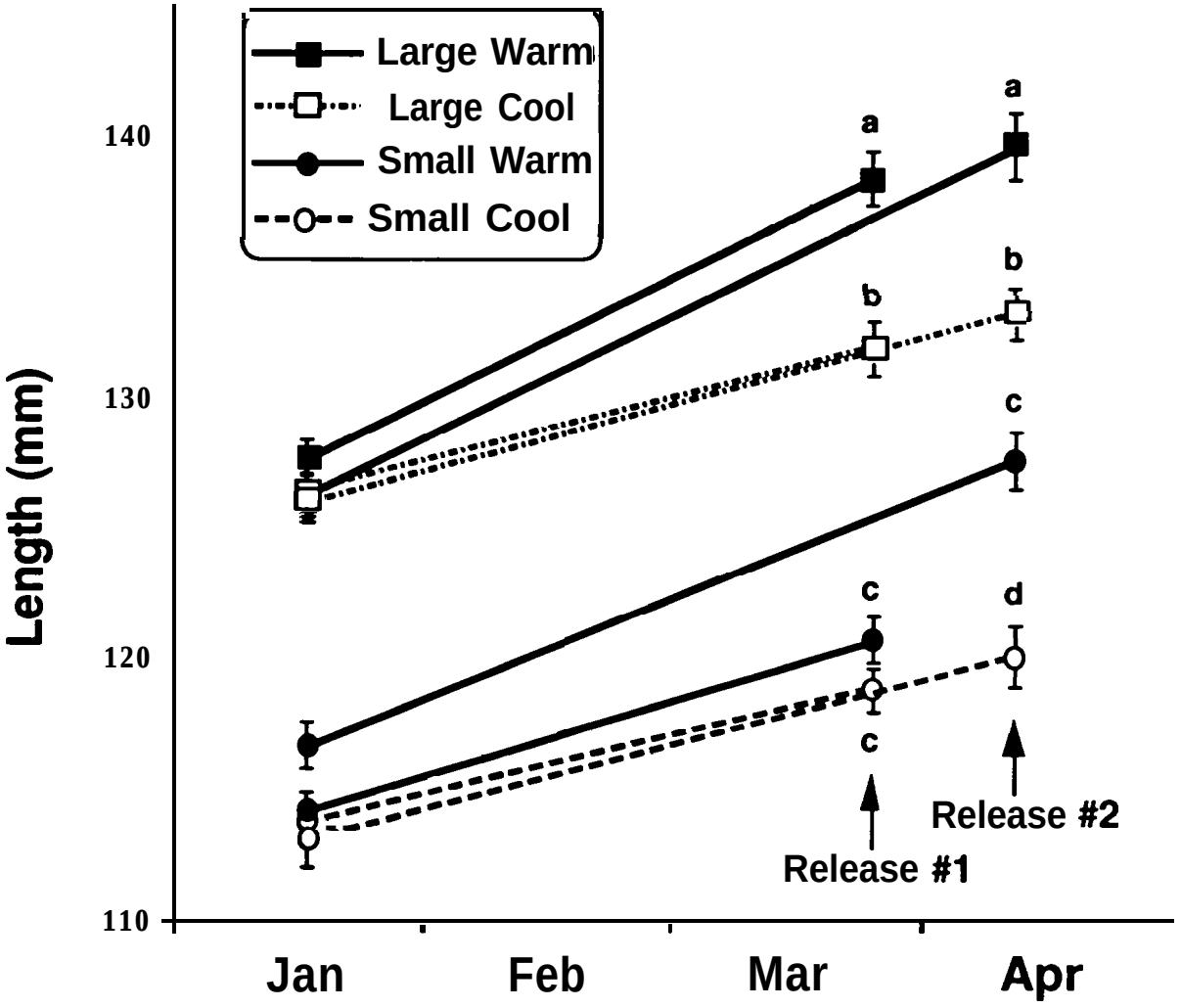

Figure 2. Fork lengths of fish from different treatment groups at tagging in January and 1 to 3 days before Release 1 (24 March) or Release 2 (10 April). Symbols indicate means; brackets indicate standard error. For each release date, symbols with different letters are significantly different (one-way ANOVA, followed by Fisher's PLSD. $P < 0.05$).

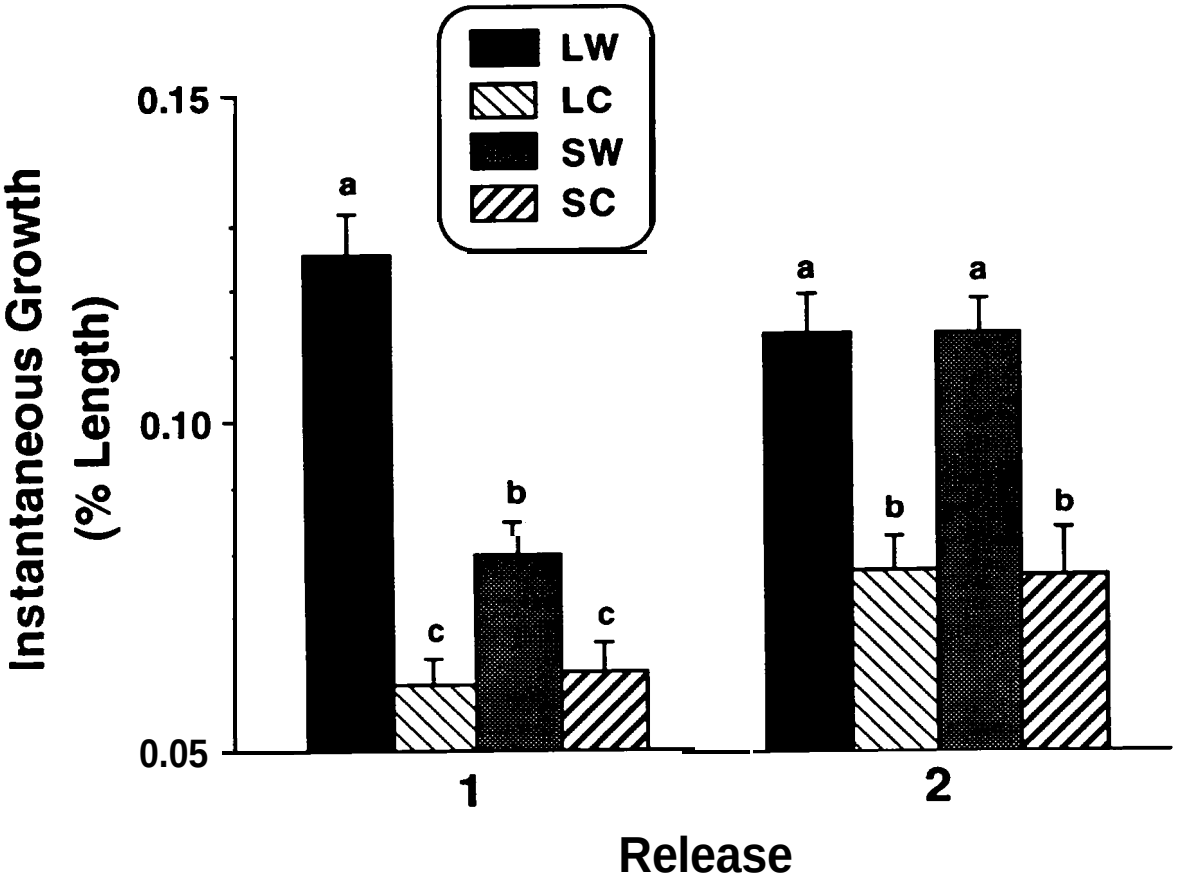

Figure 3. Instantaneous growth rates (% length/day) of treatment groups, mean + standard error, determined from 16 January to 24 March (Release 1) or 10 April (Release 2). LW = large warm-water, LC = large cool-water, SW = small warm-water, SC = small cool-water. For each release date columns with different letters were significantly different (one-way ANOVA, followed by Fisher's PLSD, $P < 0.05$).

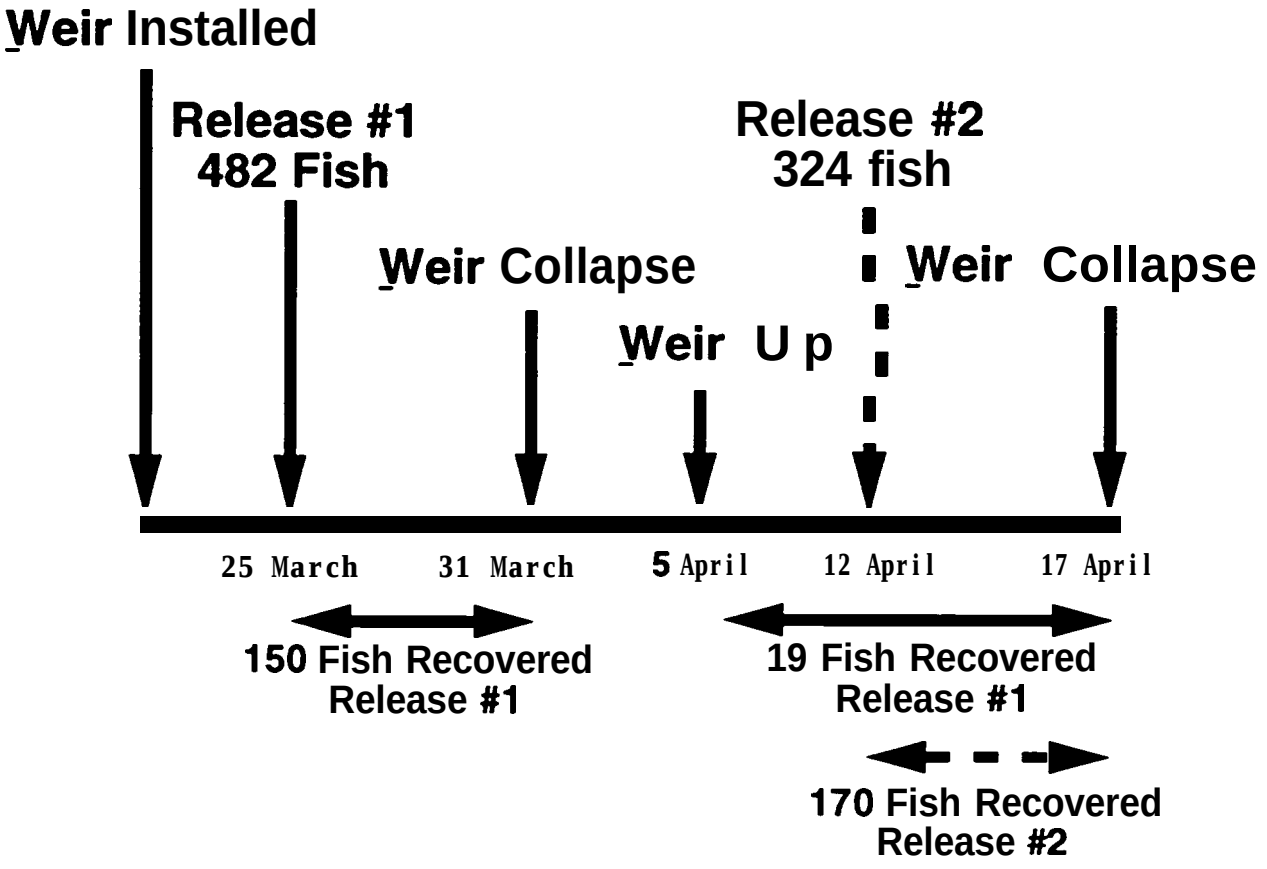

Figure 4. Chronology of fish release, monitoring, and weir integrity during 1994.

The temporal patterns of fish movement are shown in Figures 5 and 6. Fish from Release 1 were first detected on the second day after release (Fig. 5). Peak migration past the weirs occurred on the third evening post release. The majority of fish moved past the weirs between midafternoon and midnight. Midafternoon movement began as the sun passed behind canyon walls, and direct lighting onto the creek was eliminated.

Fish from Release 2 began moving past the first weir almost immediately after release, with peak migration past the first weir occurring during the first evening post release (Fig. 6). Passage through the weirs was also centered on the early evening hours. There was no obvious relation between daily fish passage and water level in the creek for either release (Figs. 5 and 6).

A comparison of migration between groups is shown in Figure 7. A chi-square test was used to examine whether there were any differences in the number of fish from each treatment group monitored at the weir prior to flooding. If there were no differences in migration between the groups, each group should have been found at the weirs in numbers proportional to their release. No difference between groups was found for Release 1 ($P = 0.46$). A highly significant difference was found for Release 2 ($P = 0.001$). Fish from both fast-growing groups (SW and LW) were found in numbers greater than expected, whereas fiih from the slow-growing groups (SC and LC) were found in numbers lower than expected.

Since PIT tags are individually coded, one may investigate the relationships between size, growth, and migration in individual fish grouped according to whether they were monitored migrating past a weir or not. For Release 1, there was no significant difference in length between early migrating (EM) and non-detected (ND) fish when they were liberated into the creek (Fig. 8; $P = 0.5$); however, EM fish were significantly larger than ND fish in Release 2 at liberation ($P < 0.001$).

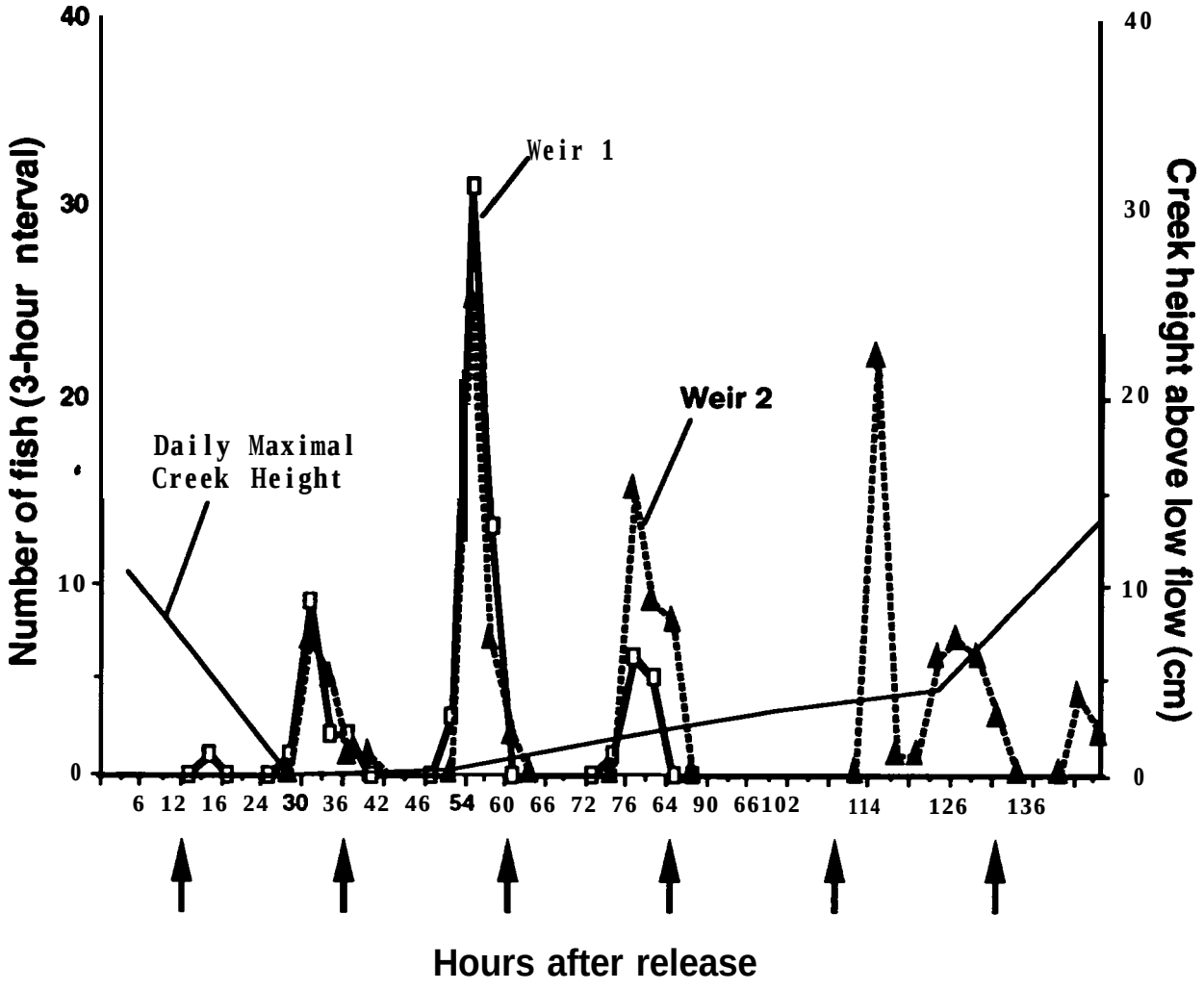

Figure 5. Temporal pattern of fiih movement through two weirs on Cooke Creek for Release 1. Release 1 occurred at 12:00 on 25 March. Passage through Weir 1 indicated by solid line with open squares, passage through Weir 2 indicated by dashed line with solid triangles. Daily creek height indicated by thin solid line (cm above low flow). Arrows below abscissa indicate midnight.

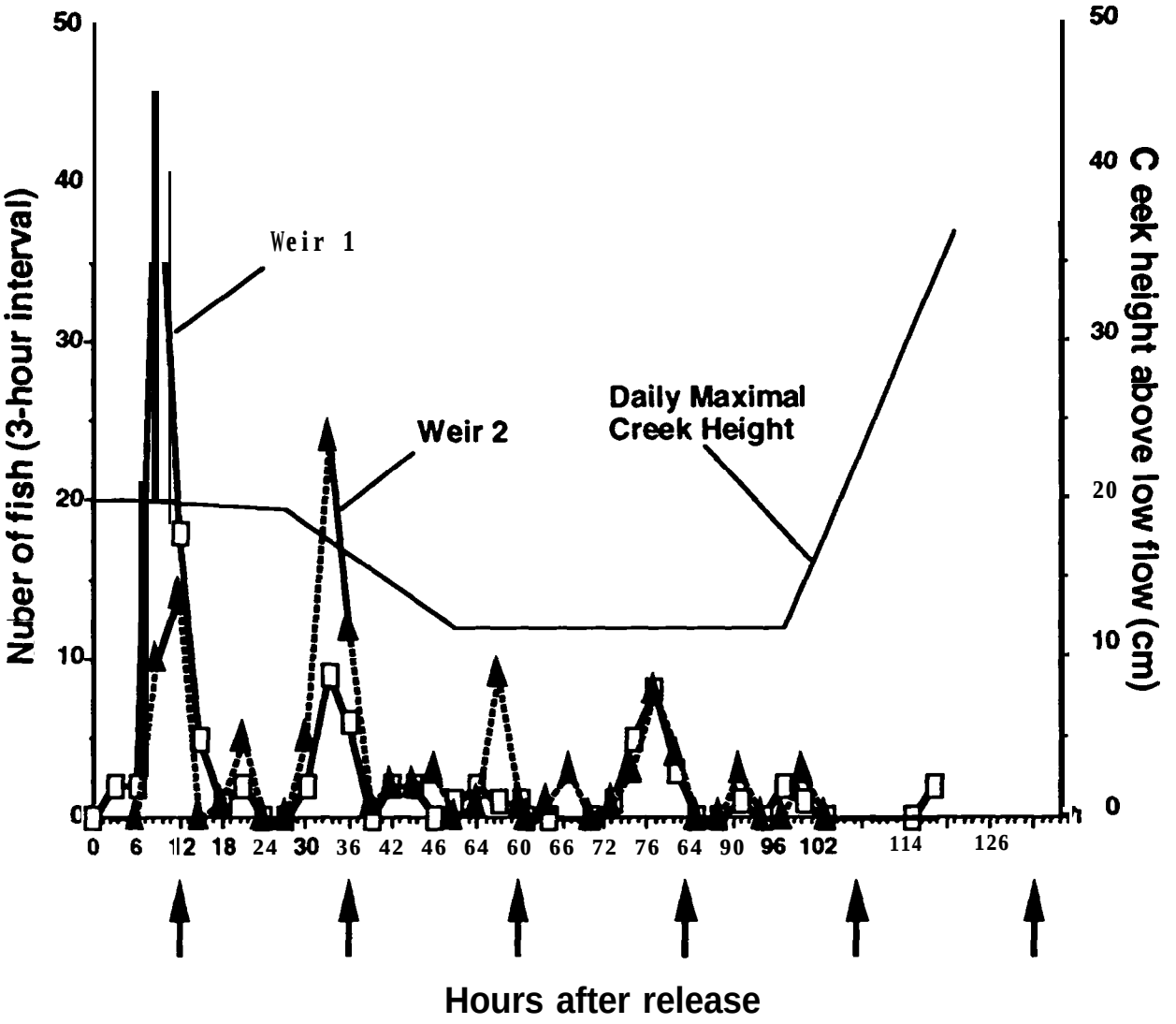

Figure 6. Temporal pattern of fish movement through two weirs on Cooke Creek for Release 2. Release 2 occurred at 13:00 on 12 April. Passage through Weir 1 indicated by solid line with open squares, passage through Weir 2 indicated by dashed line with solid triangles. Daily creek height indicated by thin solid line (cm above low flow). Arrows below abscissa indicate midnight.

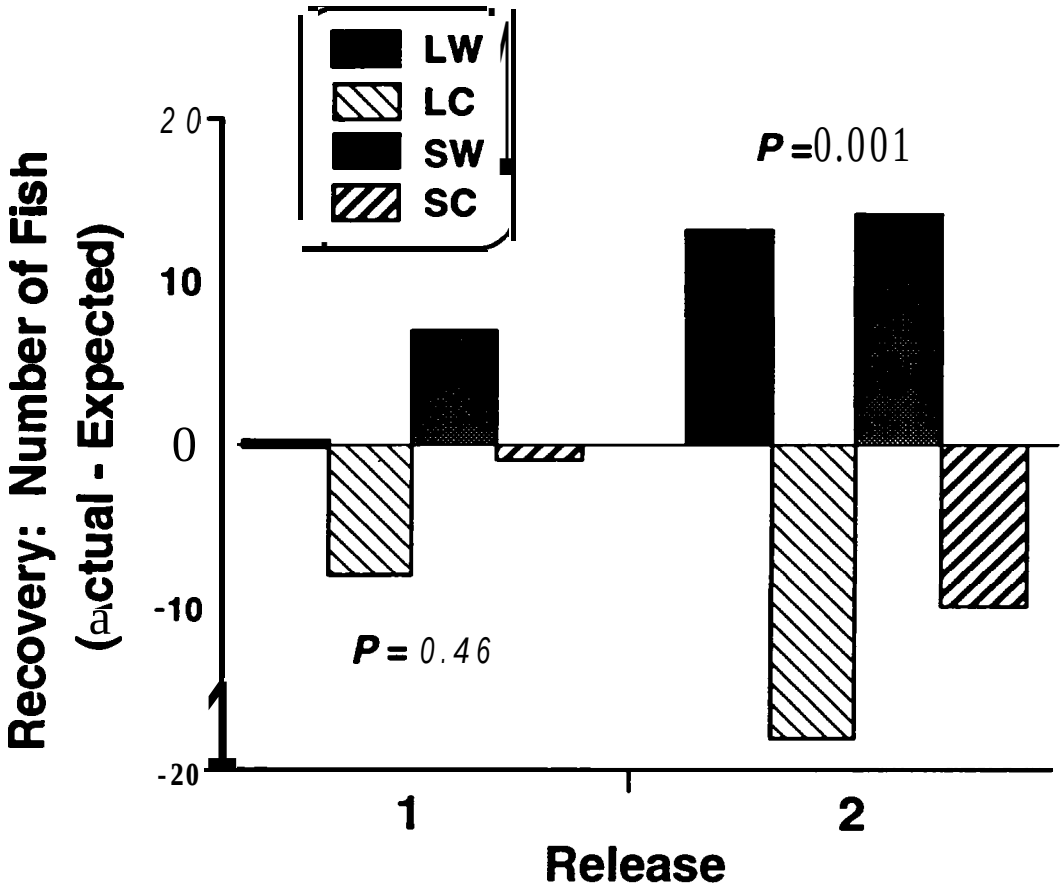

Figure 7 Recovery of fish from separate treatment groups for Release 1 and Release 2; LW = large warm-water, LC = large cool-water, SW = small warm-water, SC = small cool-water. Actual = total number of fish from given treatment monitored at either weir. Expected = number of fish from a treatment released into creek multiplied by recovery proportion for all four treatments combined.

Although there was no difference in length between EM and ND fish from Release 2 at tagging in January (P = 0.76). the EM fish were larger by the time of release and therefore had grown faster than ND fish after tagging. Calculation of individual growth rates revealed that for each release, EM fish had significantly higher average spring growth rates than ND fish (Fig. 8; Release 1 P = 0.027; Release 2 P < 0.001). Thus direct comparison of sizes between EM and ND fish from Release 2 was compromised by higher growth rates in EM fish. However, for both releases, early migrants had higher growth rates than nonmigrants. This supports evidence of a relationship between growth rate and enhanced downstream migratory movement.

A strong relationship between growth rate and migratory tendencies is found when one compares the characteristics of Release 1 fish monitored in Stanza A (passed weir prior to flooding) and Stanza B (passed weir after it was reinstalled). Fish from Stanza A had significantly higher growth rates than those from Stanza B (Fig. 9; P = 0.0002) but were not significantly different in length (P = 0.08). It also took fish from Stanza B an average of 34 hours to pass from Weir 1 to Weir 2. nearly twice as long the time required by fish from Stanza A.

On average, travel time between the first and second weirs was 19.7 and 18.2 hours for Release 1 and Release 2. respectively. This relatively long period suggests that the time taken to pass between the two weirs might be an independent measure of migration. A Spearman rank correlation test revealed a significant relation between hours to Weir 1 (W 1) and hours to transit Weir 1 to Weir 2 (W1-W2) for Release 1 (rho = 0.305, P = 0.02) and a nonsignificant relation for Release 2 (rho = 0.14, P = 0.22). This suggests that one may use W2-W 1 as an independent estimate of migration tendency only for Release 2.

The possible relation between relative size or growth rate and migratory speed (hours taken to get to a weir) for fish observed passing a weir was also examined. NO

significant relation was found for Release 1 (length vs. W1, *rho* = -0.13, P = 0.28; growth vs. W1, *rho* = -0.068, P = 0.56). For Release 2, length was not related to W1 (*rho* = -0.074, P = 0.41) and was related to W1-W2 (*rho* = -0.303, P = 0.009). Growth was significantly related to W1 (*rho* = -0.301, P = 0.0008) and W1-W2 (*rho* = -0.35, P = 0.0026). Thus we found an indication of a positive relationship between growth rate and downstream migration within the 5 days that the weir was intact after Release 2.

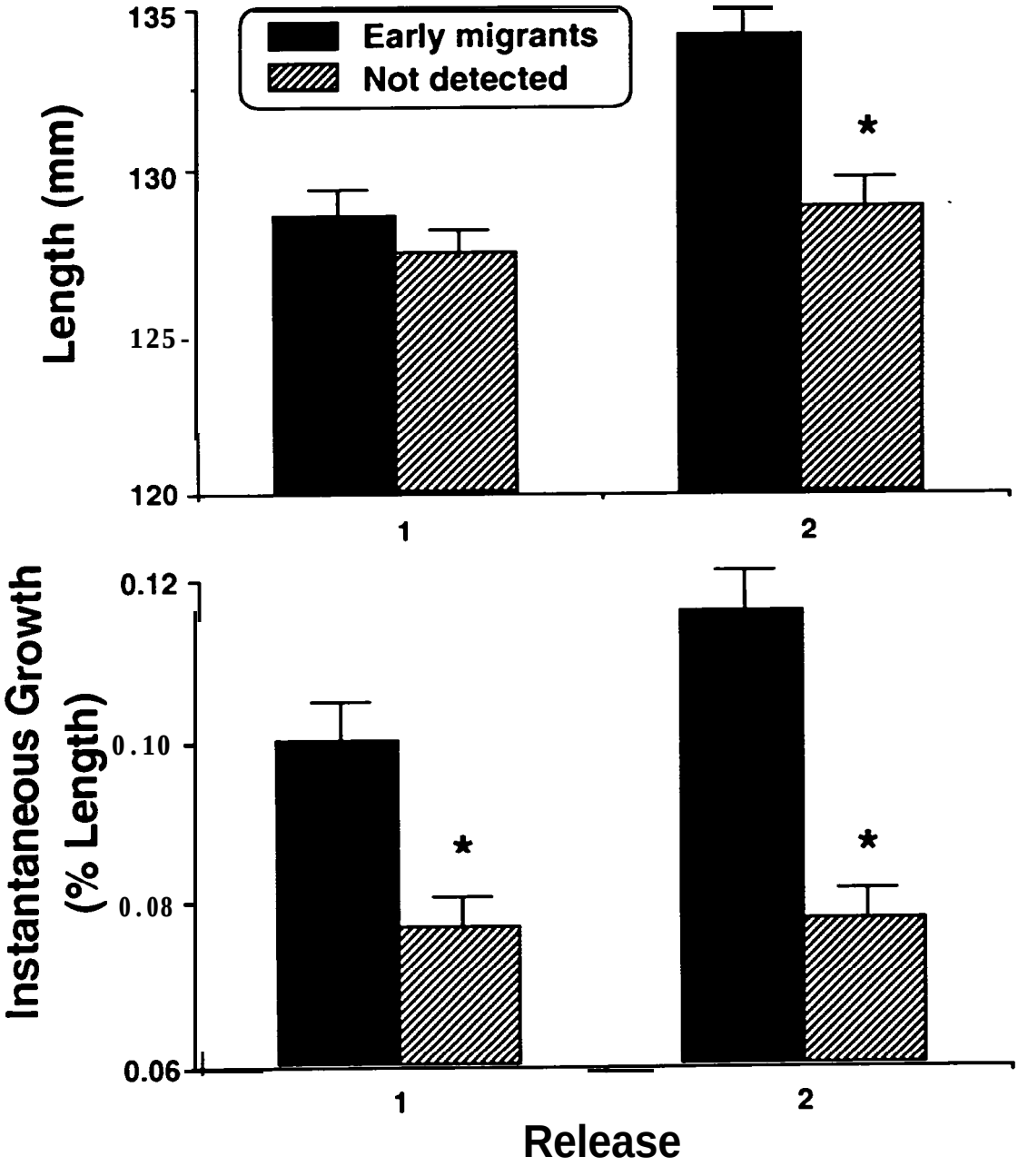

Figure 8. Length (on 24 March - Release 1 or 10 April - Release 2) and growth rate (from 16 January to 24 March - Release 1 or to 10 April - Release 2), mean + standard error, of fish released into Cooke Creek and either observed passing a weir prior to 31 March (Release 1) or 17 April (Release 2) [Early Migrants] or not detected. Significant differences between groups (one-way ANOVA, $P < 0.05$) indicated by *.

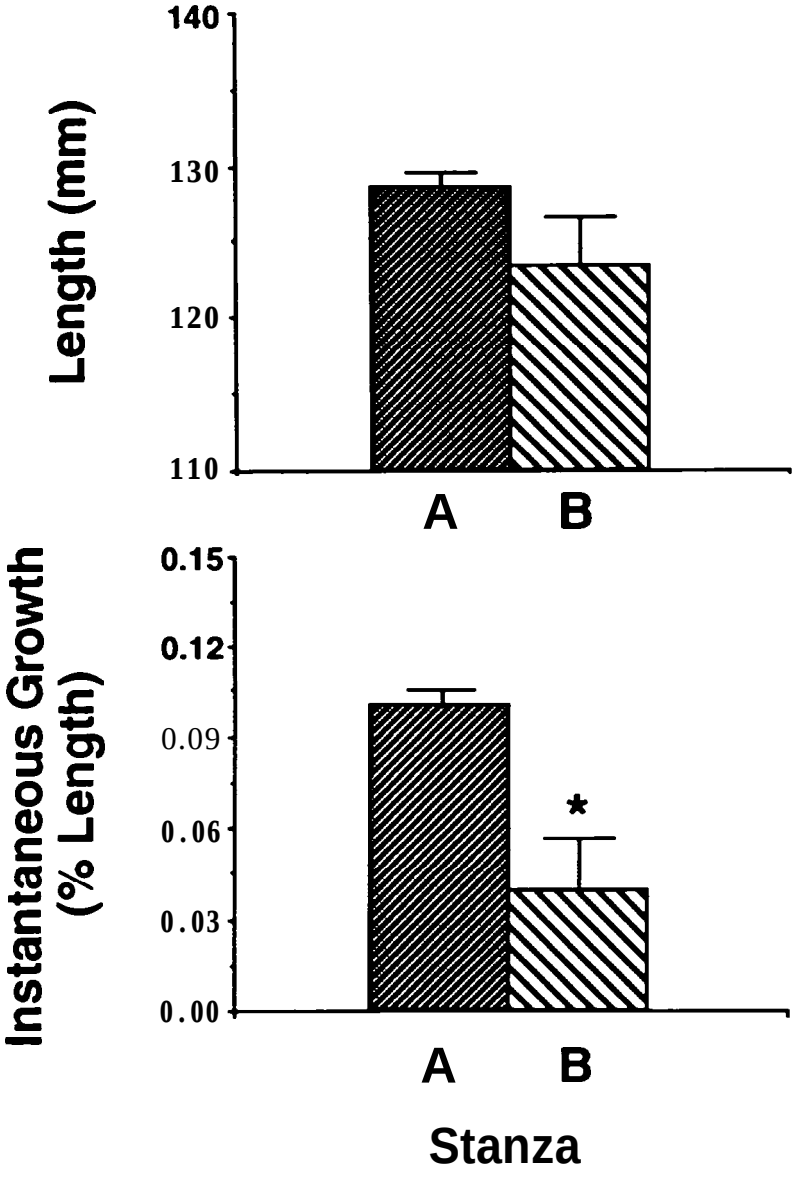

Figure 9. Length (24 March) and growth rate (from 16 January to 24 March), mean + standard error, of fish from Release 1 observed passing a weir from 25 March to 31 March (Stanza A) or from 5 April to 17 April (Stanza B). Significant differences between groups (one-way ANOVA, $P < 0.05$) indicated by *.

Physiology experiment

Significant differences in size between large and small treatment groups were found on all dates. All treatment groups increased in both length and weight from January to May (Fig. 10); growth was higher in groups maintained in the heated water (LW, SW) than in groups held at ambient temperature (LC, SC). Significant differences in length and weight were found between replicate tanks in SW. SC, and LC treatment groups (Fig. 10). Over the course of the experiment, length increased from 125 to 158 mm for the LC groups and from 125 to 172 mm for the LW groups. The SC groups grew in length from 112 mm to 140 and 145 mm. and SW groups grew from 112 and 117 mm to 150 and 155 mm. respectively.

Size differences between fish reared in different temperatures became apparent in March and April. For large fish. significant differences in size between tanks were found on 18 March. when fish from one of the LC replicates were smaller than fish from either LW tank or the second LC tank. Both LW replicates were significantly larger than both LC replicates in April and May. One of the SW replicates was significantly larger than the other small-fish groups throughout the study. Other differences became apparent on 20 April, when one of the replicates in the SC treatment was significantly lighter and shorter than the other SC tank and the SW groups. On 23 May both SW groups were significantly larger than the SC groups. A power analysis (Zar 1984) for $n = 60$, $P = 0.05$, and power = 0.9 allowed us to detect a significant difference as small as 0.5 mm between mean lengths of fish in different tanks.

Instantaneous growth (based on length) for treatment groups during four successive intervals, beginning in mid-January and ending in mid-May, are shown in Figure 11. Significant differences between date, temperature, and date by temperature in a three-way ANOVA suggested these variables had effects on growth, both seasonally

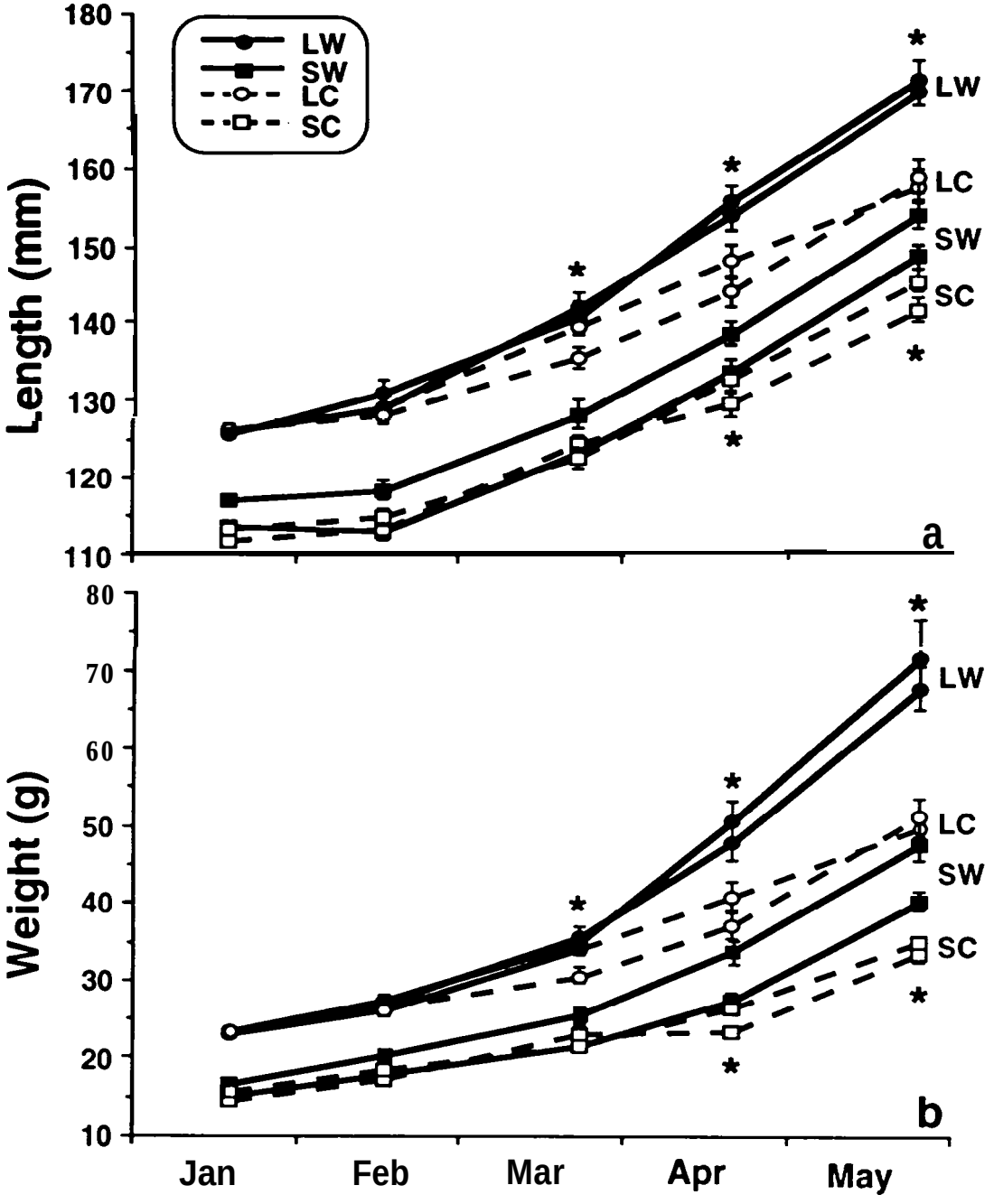

Figure 10. Length (a) and weight (b) of replicate tanks of four treatment groups: small warm-water (SW), large warm-water (LW). small cold-water (SC), large cold-water (LC). One replicate tank of the SC treatment was larger than other small size groups throughout the experiment. Asterisks indicate additional significant differences within size groups for a given date (one-way ANOVA, followed by Fisher's PLSD. $P < 0.05$).

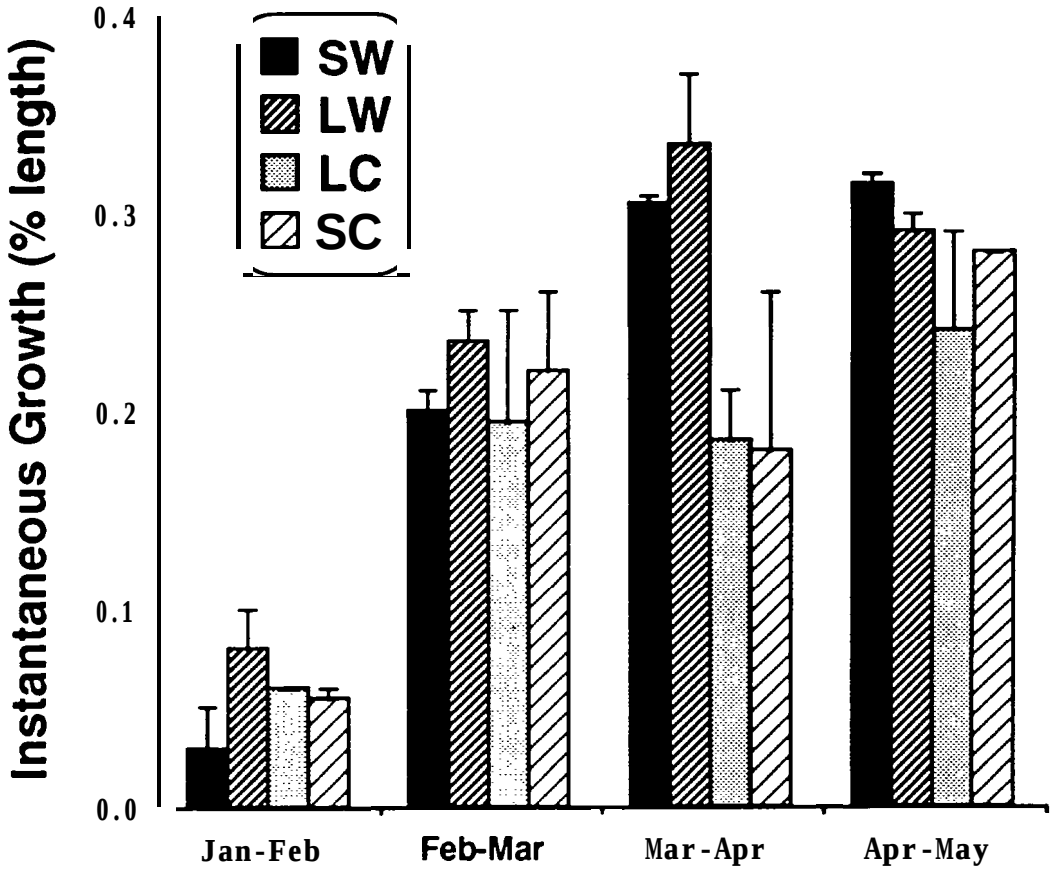

Figure 11. Instantaneous growth for fish in four treatment groups: small warm-water (SW), large warm-water (LW). small cold-water (SC), large cold-water (LC).

and between treatments. Growth rate for all groups doubled from January-February to February-March. but there were no clear significant differences among the different experimental groups for the two periods. Growth rate for LW and SW groups increased again in March-April. whereas rates LC and SC groups remained similar to those observed in February-March. Growth rates for all groups were similar in the April-May period.

Plasma IGF-I increased significantly between late January and mid-February; values rose from about 70 to greater than 85 ng/mL (Fig. 12). Both SW and LW treatments showed a further significant increase in mid-March to values greater than 110 ng/mL. In mid-March significant differences between treatments were found. and these differences were maintained through May, with IGF-I levels consistently higher in the warm-water groups. There was little apparent effect of size on plasma IGF-I level. There were no differences in IGF-I levels between LC and SC treatments. LW fish had significantly higher IGF-I levels than SW fish in only two (late March and late May) of the nine dates measured. For all five physiological parameters examined, significant differences between replicate tanks within a treatment were only found for SC fish for body lipid. Accordingly. all replicate values were pooled within treatments.

Plasma thyroxine (T4) levels increased significantly from late February to May in all groups (Fig. 13). There were no significant differences between treatment groups on any one date.

Gill Na^{+}-K^{+} ATPase activity increased significantly in all groups to reach peak values in late April (Fig. 14). The only significant difference between treatment groups was observed in late February, when the LW group had higher levels than the other groups.

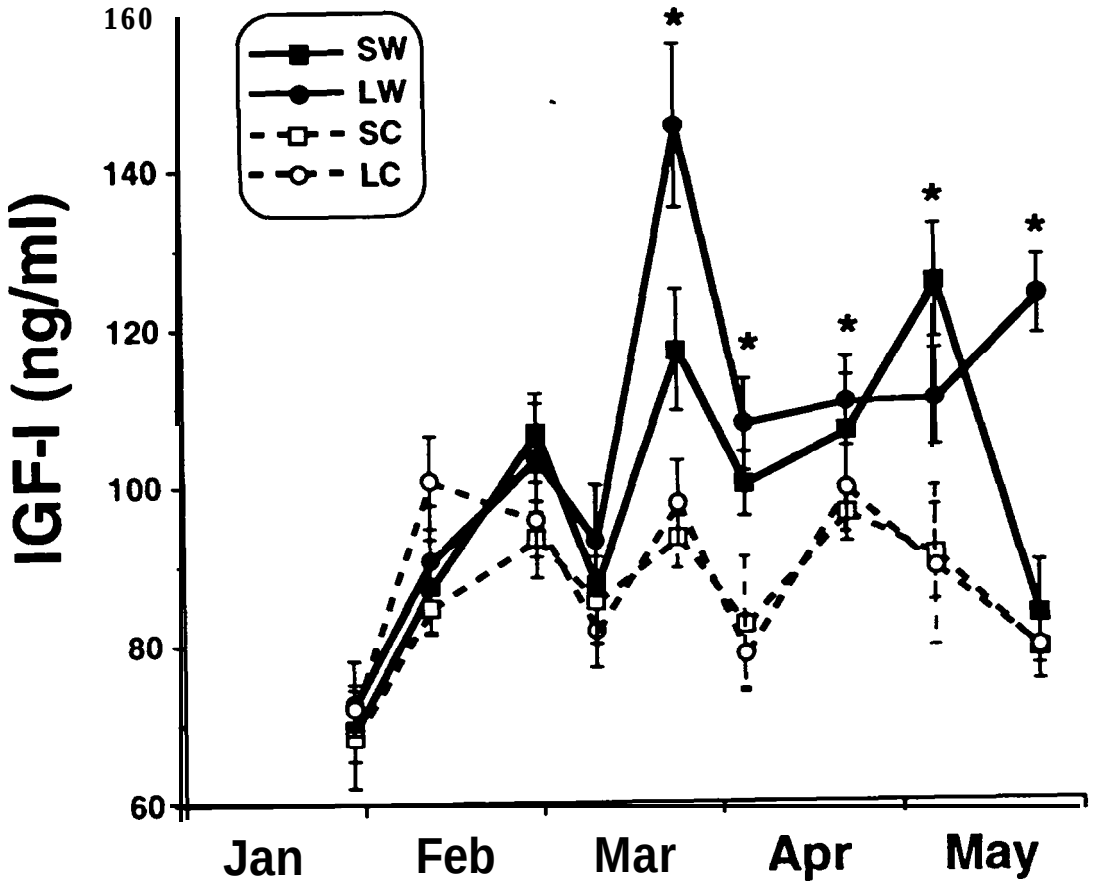

Figure 12. Plasma insulin-like growth factor-I (IGF-I) concentrations in four treatment groups: small warm-water (SW), large warm-water (LW), small cold-water (SC), large cold-water (LC). Asterisks indicate significant differences between treatment groups for a given date (one-way ANOVA, followed by Fisher's PLSD, $P < 0.05$).

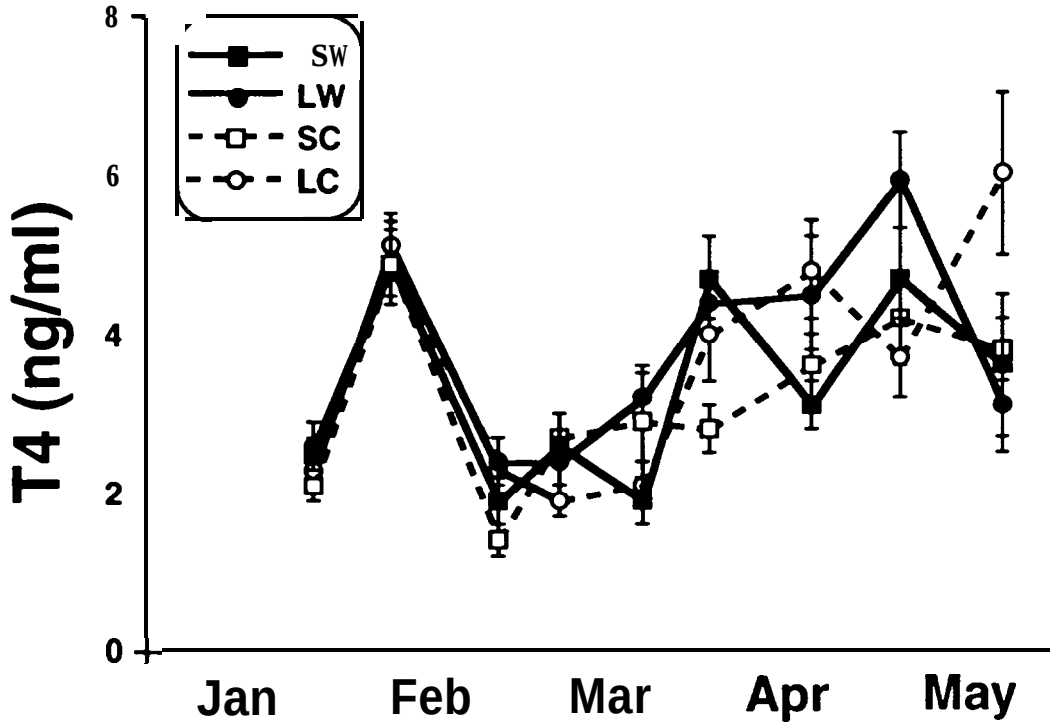

Figure 13. Plasma thyroxine (T4) concentrations in four treatment groups: small warm-water (SW). large warm-water (LW). small cold-water (SC), large cold-water (LC).

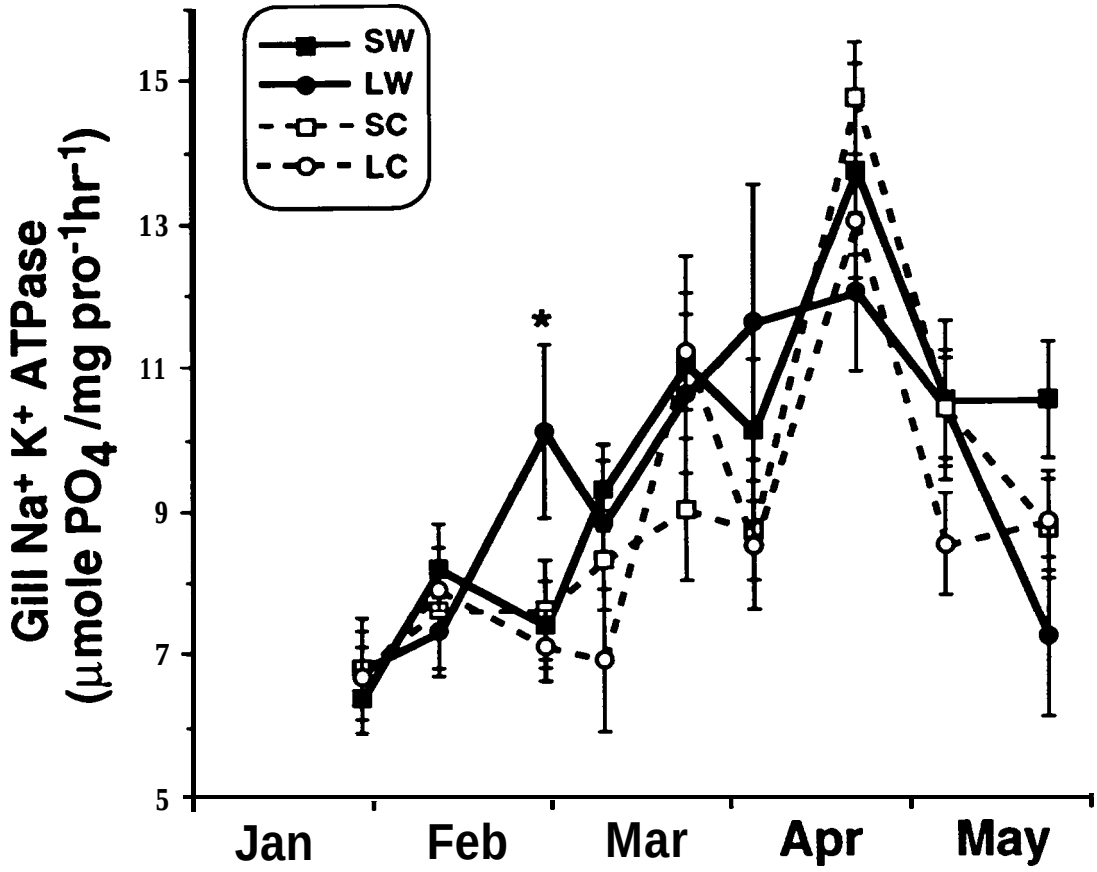

Figure 14. Gill Na^+ K^+ ATPase activities in four treatment groups: small warm-water (SW). large warm-water (LW), small cold-water (SC). large cold-water (LC). Asterisk indicates a significant difference between treatment groups for one date (one-way ANOVA, followed by Fisher's PLSD, $P < 0.05$).

Liver glycogen concentrations for all treatments were highest in January-February, declined from February to the end of March, and then remained relatively low through April and May (Fig. 15). Significant differences between treatment groups were found on only one date in April. when glycogen levels in LC fish were significantly lower than those in the other treatments.

Whole body lipid levels were relatively constant in most groups over time (Fig. 16). Only the LC group showed a significant decline from initial body lipid levels. In late February, LW fish had higher lipid levels than either cool-water group. and this was the only time when significant differences were found among treatment groups.

A highly significant ($P < 0.001$, $R^2 = 0.69$) positive relationship was found between mean IGF-I level in a tank during a growth interval and instantaneous change in length for that interval (Fig. 17). A similar comparison between mean plasma T4 levels in a growth interval and instantaneous change in length showed no apparent relation ($P = 0.59$, $R^2 = 0.009$).

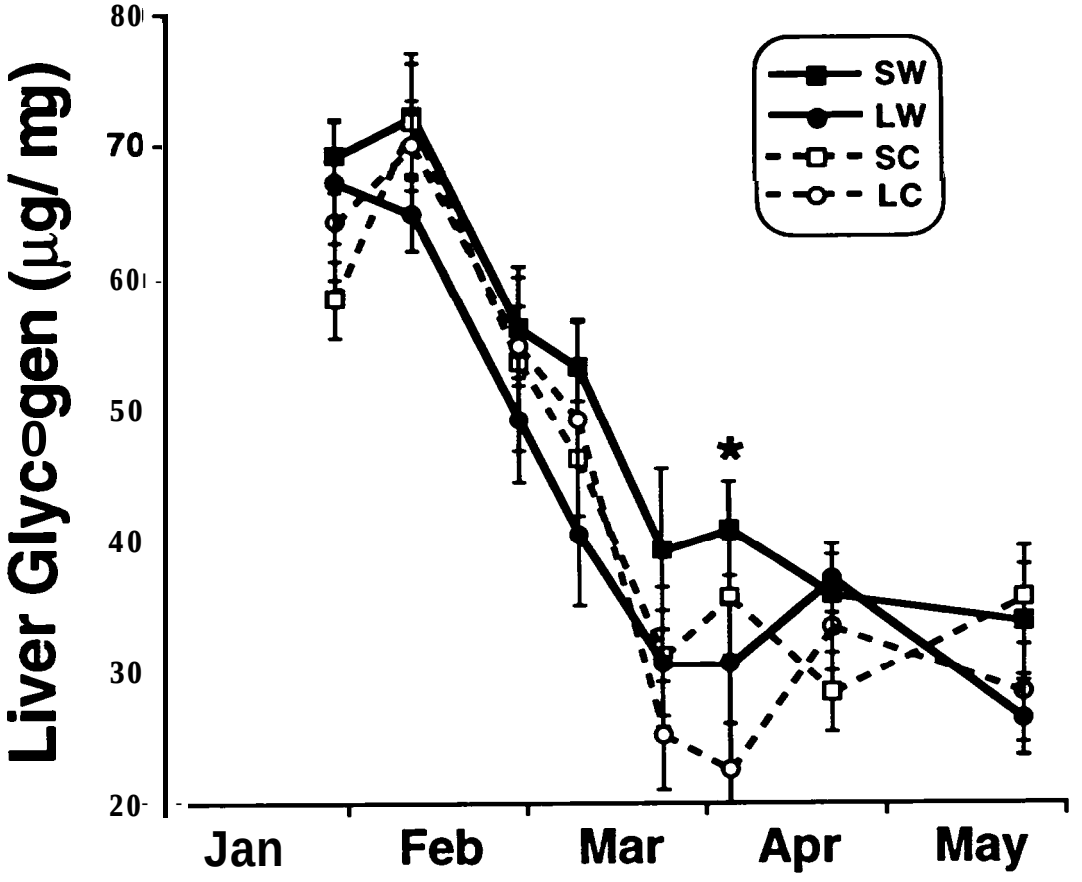

Figure 15. Liver glycogen levels in four treatment groups: small warm-water (SW), large warm-water (LW), small cold-water (SC), large cold-water (LC). Asterisk indicates a significant difference between treatment groups for one date (one-way ANOVA, followed by Fisher's PLSD, $P < 0.05$).

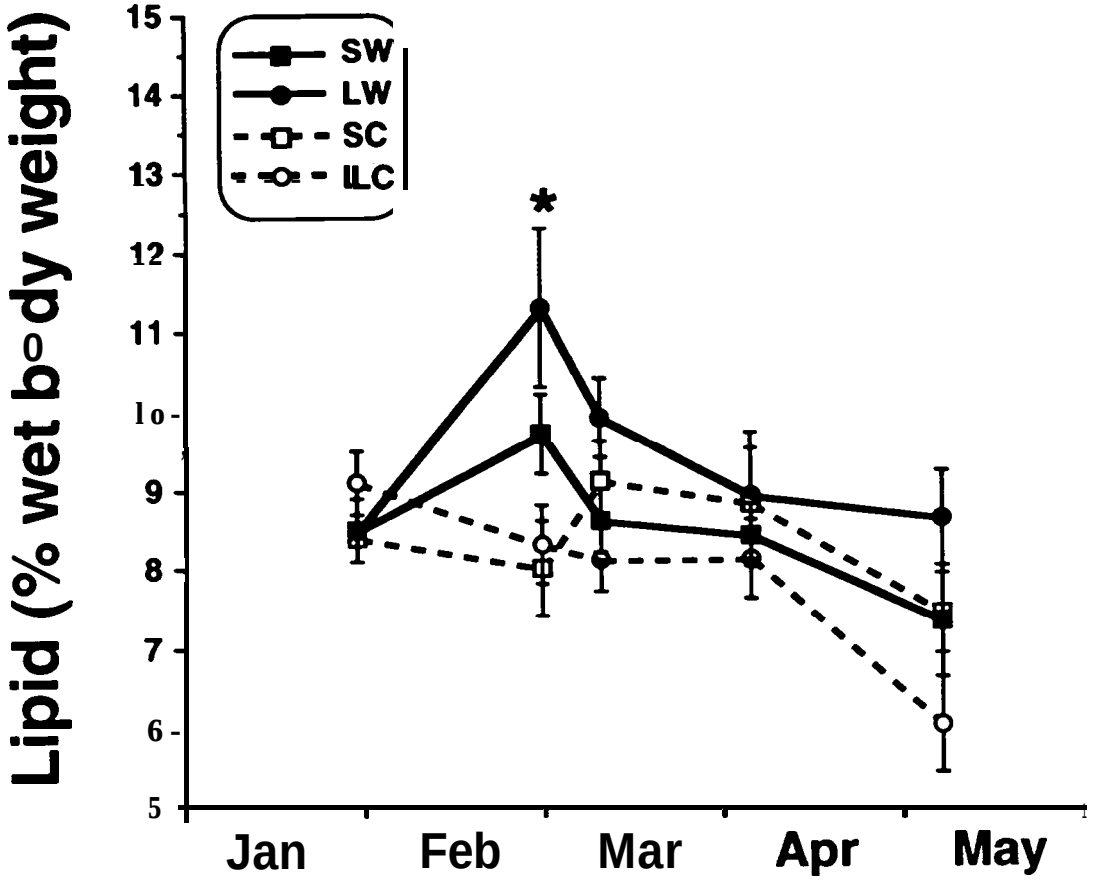

Figure 16. Whole body lipid (% wet weight) in four treatment groups: small warm-water (SW), large warm-water (LW), small cold-water (SC), large cold-water (LC). Asterisk indicates a significant difference between treatment groups for one date (one-way ANOVA, followed by Fisher's PLSD. $P < 0.05$).

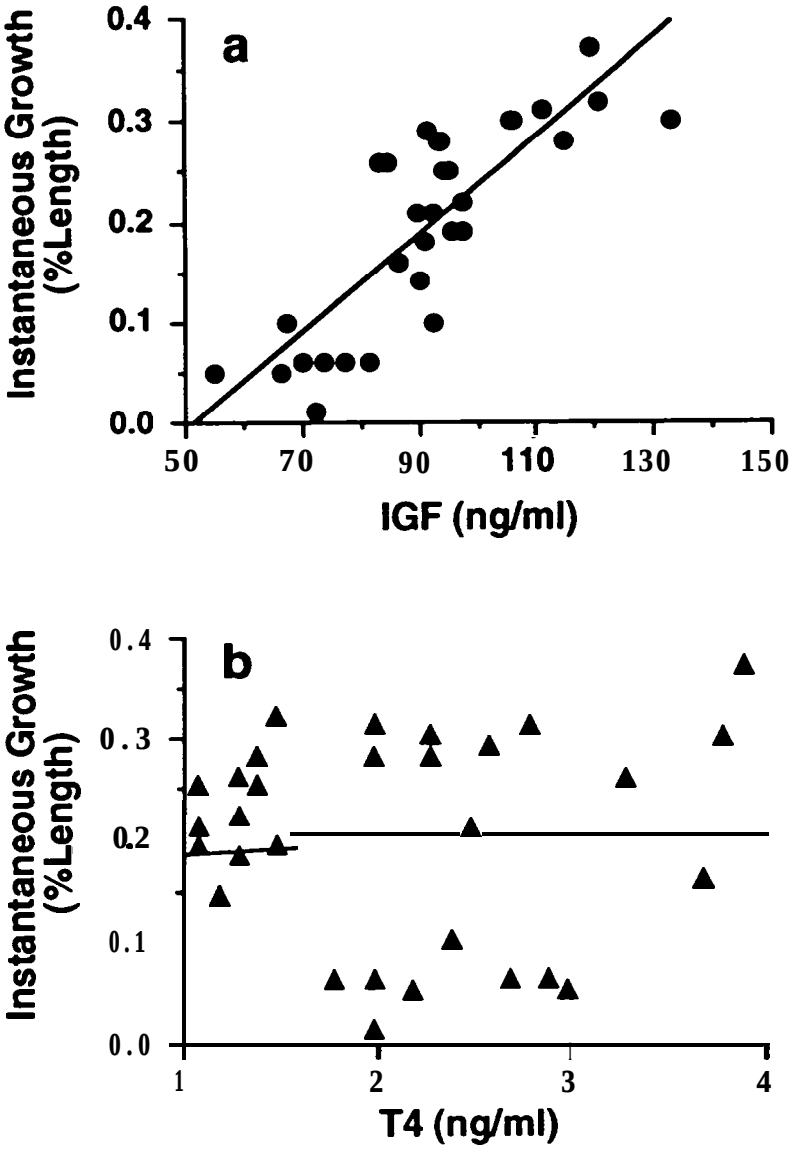

Figure 17. Relationship between (a) instantaneous growth (% length) and plasma insulin-like growth factor-I (IGF-I) ($P < 0.001$, $R^2 = 0.67$) or(b) plasma thyroxine (T4) ($P = 0.59$, $R^2 = 0.009$). Each point represents the mean plasma hormone level found in a tank for a given growth interval (Jan-Feb, Feb-Mar, Mar-Apr. Apr-May) generated from data shown in Figure 9.

DISCUSSION

These experiments were designed to discriminate the relative influence of size and growth rate to smoltification as assessed by migratory behavior and physiological indices. Four distinct treatment groups were created: large and small fish, each growing at relatively fast or slow rates. Our hypothesis was that the generally accepted belief that fish body size affects smoltification may be more of a reflection of recent growth history than body size per se. Studies of the effect of smolt size on smolt-to-adult survival have generated and maintained size differences by manipulating growth rates. For example, Martin and Wertheimer (1989) compared smolt-to-adult survival of small fish fed a reduced ration rate and large fish fed an increased ration. Fish released as large smolts returned at a twofold higher rate than small smolts. Similarly, Bilton (1984) manipulated growth to generate size differences in groups of underyearling chinook salmon. They also found that large fish survived to adulthood at a higher rate than small fish.

In retrospect, these experimental designs did not test the effects of size alone, rather they tested the combined effects of growth rate and size on survival. The strength of our experimental design is that it allows the explicit examination of the relation of growth to the variable of interest without confounding the effects of size. Our findings show that migratory tendency and plasma IGF-I levels during smoltification are influenced by growth rate and not body size, at least within the range of growth rates observed.

In the experiment on migration we used three separate analyses to demonstrate a positive relation between migration of smolting chinook salmon and growth rate in the spring; average growth rates of early migrating fish were greater than those of nondetected fish. For Release 2, we recovered a higher proportion of fish from fast

growing groups than from slow growing groups. In addition, we found a positive relation between migratory speed and growth rate for fish from Release 2.

A positive relationship between size and migration was also demonstrated, although the relationship was weaker for size than for growth rate. It was difficult to discriminate the relationship between growth and size in comparisons of early and nonmigrating fish. since fish that had experienced rapid growth in the spring became larger than fish that grew at a lesser rate. However, for Release 1 large fish were not observed at a weir in greater numbers than small fish. In Release 2, small fast-growing fish moved faster than large slow-growing fish, as they were caught at higher relative proportions at the weir. Overall, size seemed relatively less important than growth in determining migratory performance, at least as migration was defined in this study.

This study was not designed to simulate natural movement of smolts out of a stream reach; rather it was designed to measure differences in migration between different treatment groups. Transportation stress and crowding, followed by introduction to a novel habitat, may have influenced fish movement The release and weir sites were selected such that there were a number of pools upstream from the first weir capable of holding fish. In selecting this type of release environment, we hoped that stress-induced movement would occur upstream from our monitoring site, leaving only smolt-related movement to be measured at the weir.

Since downstream movement was linked to growth, as hypothesized, we were able to discriminate downstream movement associated with smoltification. This assertion is strengthened by the observation that downstream movement was faster and more prevalent in fish of Release 2, which may have been closer to their peak of smolt development. We found peak $Na^+ K^+$ ATPase values in mid-April for the fish in the physiology study. Displays of downstream movement would be expected to be heightened later during the smolting period, coincident with peak $Na^+ K^+$ ATPase activities (Zaugg 1981). Our two tests of migration were not replicates. Rather, they

were conducted at different points in relation to smolt development; accordingly, different results might be expected.

In the physiology experiment except for plasma IGF-I levels, we found no major treatment effects on the physiology of smolt development. Liver glycogen concentration, gill ATPase activity, and plasma T4 levels all showed the expected changes coincident with smoltification (Zaugg 1981, Dickhoff et al. 1982, Sheridan et al. 1985). although they were similar among treatment groups. It is important to note that the slow-growing fish in our experiment actually had a relatively greater growth rate than seen in some hatcheries (Dickhoff et al. 1995). All treatment groups had relatively high growth rates. Dickhoff et al. (1995) observed clear physiological differences in timing and intensity of ATPase development and differences in peak T4 values; however. they studied slow-growing groups in which there was little or no size change through the spring.

We found no relation between size and smolt physiology or migratory disposition, even though there was a twofold difference in weight between treatment groups. Similarly, Dickhoff et al. (1995) found little relation between physiological development and smolt size. Many studies have found a relation between hypo-osmoregulatory ability of smolts and size. Since growth hormone (GH) has clearly been shown to stimulate hypo-osmoregulatory ability (Komourdjian et al. 1976a). it is difficult to determine whether the underlying mechanism of hypo-osmoregulatory development is related to size (large fish must have grown faster than relatively small fish) or growth. We did not directly measure hypo-osmoregulatory ability but found no difference in Na+-K+ ATPase activity between different size groups. Our results suggested that physical size alone had little effect on physiological development.

This report is the first to show changes in circulating levels of IGF-I during the parr-smolt transformation using a recently developed homologous radioimmunoassy developed by Moriyama et al. (1994). Lindahl et al. (1985) used a mammalian radioreceptor assay to show a seasonal peak of plasma IGF-I levels associated with

smoltification in Atlantic salmon (Sulmo *salar).* **Duguay** et al. (1994) showed an increase in liver and gill IGF-I mRNA in smolting coho.

The IGF-I results are significant in two respects: a) a potential specific role of IGF-I in smoltification, and b) the relation of IGF-I to somatic growth in juvenile salmon. Endocrine control of the parr-smolt transformation involves the growth hormone (GH)-IGF-I axis, thyroid hormones, cortisol, and insulin. among others (Dickhoff 1993). Administration of various hormones to stimulate smoltification has suggested that GH is most effective (Miwa and lnui 1985). Furthermore, it is well established that circulating levels of GH increase during smoltification (Sweeting et al. 1985. Young et al. 1989. Prunet et al. 1989, Boeuf et al. 1989, Schmitz et al. 1994). Stimulation of smoltification by increasing photoperiod is most likely mediated by GH (Komourdjian et al. 1976b. Björnsson et al. 1989. Okumoto et al. 1989, McCormick et al. 1995).

Many of the actions of GH are mediated by IGF-I produced in peripheral tissues. In salmon. the majority of circulating IGF-I comes from the liver (Duan et al. 1994). IGF-I is also produced in most other salmon tissues (Duguay et al. 1994) where they apparently have autocrine/paracrine effects and, for the most part. do not seem to be responsive to GH. In addition to the liver, other tissues that produce IGF-I in response to GH may include gill and kidney (Sakamoto and Hirano 1993).

The increase in hypo-osmoregulatory ability of salmon during smoltification appears to be mediated in part by GH, since GH treatment results in increased hypo-osmoregulation upon transfer to seawater (Komourdjian et al. 1976b. Clarke et al. 1977, Miwa and lnui 1985. Bolton et al. 1987. Collie et al. 1989. McCormick et al. 1991, Boeuf et al. 1994). Some of the osmoregulatory actions of growth hormone are mediated by IGF-I (Sakamoto et al. 1993). Thus. increases in IGF-I during smoltification may have a specific function in promoting preadaptive salinity tolerance. The relative roles of circulating IGF-I versus IGF-I produced locally in gill or other osmoregulatory tissues remain to be established.

Our data did not show a clear pattern of IGF-I change in relation to other physiological parameters of smoltification, although a significant increase in IGF-I was found between January and February in all treatment groups. The LW and SW fish showed a second increase in IGF-I in mid-March. Plasma IGF-I levels in SC and BC treatment fish stayed at a relatively constant level from February through May. Based on other measured physiological parameters, and in comparison to the LW and SW treatment groups, the LC and SC groups showed a typical pattern of smolt development. The relatively few reports of IGF-I levels in smolting salmon (Lindahl et al. 1985, Duguay et al. 1994, Moriyama et al. 1994) suggest that there is a smoltification-associated rise in IGF-I. None of these reports measured plasma IGF-I in chinook salmon. We are unable to judge if the January-February increase in IGF-I found in LC and SC fish is of comparable magnitude to IGF-I increases found in other smolting salmon. Thus, we were unable to ascertain a "typical" pattern of IGF-I change in plasma in these fish during smoltification.

However, the relation of plasma IGF-I and somatic growth is becoming clear. Our relatively fast-growing fish showed elevated levels of IGF-I compared to slow-growing fish. Regression analysis suggested that much of the seasonal variation in growth within our treatment groups was explained by differences in IGF-I during each growth interval. This was in contrast to T4 levels, which showed no relation to growth in an identical analysis. Within the limits of our experimental data, the regression analysis suggested a concentration-response relationship for plasma IGF-I levels and somatic growth: higher levels of IGF-I lead directly to greater growth.

The relationships of IGF-I to GH and somatic growth have been recently established in fish. McCormick et al. (1992) showed that IGF-I treatment resulted in increased growth in coho salmon. Low growth rate (stunting) of coho salmon in seawater is associated with low levels of hepatic IGF-I mRNA (Duan et al. 1995). Feeding and fasting and manipulation of growth rates by ration and protein intake results in a good

correlation between circulating IGF-I and growth rate in gilthead seabream *(Spurus aurutu)* (Perez-Sanchez et al. 1994.1995).

In addition, changes in plasma IGF-I levels appear to precede changes in somatic growth. There was a clear lag between the mid-February increase in water temperature for the LW and SW treatment groups and the significant increase in plasma IGF-I in mid-March. Similarly, somatic growth between treatment groups only differed in the March-April interval; no difference in growth was found in February-March. the interval when temperature treatment was begun. Although Duan and Plisetskaya (1993) and Duan et al. (1994) observed rapid response to hormonal and nutritional treatments in liver IGF-I m-RNA levels. the lag in response found in our results suggested that a significant period of time is needed to recruit the endocrine mechanism which results in increased plasma IGF-I and somatic growth.

The importance of growth rate in animal development has long been recognized in insects (Beck 197 1). amphibians (Wilbur and Collins 1973). and fish (Policansky 1983, Stearns 1983). High growth rate may accelerate or delay metamorphosis depending on several environmental factors (cf. Bradshaw and Johnson 1995). The parr-smolt transformation of juvenile salmonids is a metamorphic-like developmental process. a "second metamorphosis" as described by Youson (1988). This is in contrast to the irreversible first metamorphosis characteristic of flatfish, for example.

No published studies of smoltification have directly examined the influence of growth rate independent of body size, although several have suggested a specific role of growth rate. However, in a study of development of osmoregulation in chinook salmon, Wagner et al. (1969) stated that "small fish which exhibited a faster growth were better regulators than larger fish which are slower growing." Analysis of growth rate by RNA-DNA ratios in coho salmon migrating through the **estuary** indicated that fish moving quickly into seawater were the fastest growing segment of the population (Varanavskiy et al. 1992). In a study of photoperiod and temperature influence on smoltification Clarke

et al. (1978) noted that “smolting is not a cumulative process, since it is influenced more by present growth rate than by past growth achieved.” An increasing growth rate during smoltification fits with the pattern found in wild salmon, based on our studies of juvenile spring chinook salmon (Beckman, Larsen, and Dickhoff unpubl. data).

In discussing factors influencing smoltification, Hoar (1976) identified priming factors (photoperiod) and releasing factors (temperature, flow or others). Increasing spring photoperiod results in an overall change in behavioral tendencies, priming the migrational urge during a distinct seasonal period. We do not believe that high growth rate alone led to increased migrational tendencies. Rather, increased growth rate acted synergistically with the increasing photoperiod naturally found in the spring. We would not expect induction of downstream migration by increased growth rate at other times of the year.

There is no readily apparent, intuitive relationship between growth rate, physiological smolt indices, and downstream migratory disposition in juvenile salmonids. However. several studies have shown that physiological smolt development and development of downstream migratory tendencies are correlated. Zaugg and Wagner (1973) showed that an advanced photoperiod in the spring induced accelerated smolt development (as measured by increases in gill Na+-K+ ATPase activity) in steelhead trout (0. mvkiss). The physiological advance was accompanied by greater downstream movement. Hart et al. (198 1) found a correlation between Na+-K+ ATPase activity and migratory disposition in hatchery chinook salmon. Muir et al. (1994) showed that advanced photoperiod and increased rearing temperatures accelerated smoltification and downstream migratory movements in hatchery-reared spring chinook salmon. These studies provide no direct evidence of a coupling of physiological change and migrational behavior. However, they do suggest a tight temporal link between physiological change and migration. Moreover, they indicate that environmental manipulations that affect physiological development also affect changes in migratory behavior.

Numerous observations of wild fish at downstream counting weirs have suggested that large smolts migrate downstream sooner than small smolts (Irvine and Ward 1989). This has led to the conclusion that large fish show increased migratory dispositions sooner than do small fish (Bohlin et al. 1993). Our results cast these observations into a new light and consequently pose the question: Do large early migrants experience higher growth rates than late migrants in natural streams? It is likely that large fish are competitively superior to small fish (Fausch 1984). Large fish might consume a disproportionate amount of early spring food, leading to a relatively earlier increase in spring growth rate than found in small fish.

The distinct seasonality of migration found in yearling smolts suggests that there is an adaptive benefit to the timing of their movement (Hoar 1976, Bilton et al. 1982). It is unclear whether there are predictable benefits to migration in the early or late part of this migration window. Experimental hatchery releases show that survival from smolt to adult is highest when fish are released in the spring (April-June), but it is unpredictable from year to year which releases made within this period will result in the highest survival (Zaugg 1989, Lundqvist et al. 1994). Thus, there may be no easily defined advantage to migrating either early or late within the spring migration period. Early migration by large fish may be simply a result of their greater ability to capture food (Fausch 1984). As such. this behavior could not be described as adaptive. Rather, an increased ration leads to increased growth rate, which inevitably leads to increased migratory tendencies.

Temperature absolutely controls growth rate in fishes (Brett 1979). Holtby et al. (1989) showed a strong positive relation between interannual spring stream temperature and median migration timing of coho salmon smolts. Relatively higher spring stream temperatures would result in higher potential growth rates in juvenile salmonids. Based on our results, the higher growth rate could result in earlier recruitment of the endocrine mechanisms which lead to downstream migratory behavior.

We observed a relatively strong effect of growth rate on downstream migratory tendency, in contrast to its relatively modest effects on physiology, and this may be significant for both smolt biology and hatchery management. Rapid and directed downstream migration is a most essential element of smoltification. Stimulation of growth of hatchery-reared salmonids during the parr-smolt transformation may improve smolt quality by 1) improving downstream migration and 2) improving seawater tolerance through stimulation of the GH-IGF-I axis. We suggest that hatcheries do not focus on absolute size as a criterion for fish production. It may be more advantageous to develop production schemes which emphasize achieving a high rate of fish growth prior to release.

However. it is unclear whether simple increases in ration at a constant temperature would also have a stimulatory effect, especially as many hatcheries already feed rations designed to produce optimal growth. It is clear that many hatcheries currently have a limited capacity to elevate water temperatures during the spring period. Also, many hatcheries, at least in the Columbia River Basin, release fish prior to natural spring increases in water temperature (Dickhoff unpublished). A larger-scale test of the relative effects of water temperature and feeding rate and their subsequent effects on growth and smolt performance is warranted.

Acknowledgments

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